Programmed cell death ligand 1 (PD-L1) (CD274, B7-H1) is the most important immune checkpoint protein in immune defense against tumors. PD-1/PD-L1 inhibitors are considered an option in cancer treatments. The evaluation of PD-L1 immunohistochemical staining is used as a biomarker to determine the decision and response of the use of these inhibitory drugs. There is a wide variety of clones and platforms for the PD-L1 antibody, and each pathology department uses different clones and platforms which causes confusion. Therefore, in this study, we evaluated the immunohistochemical staining of different clones in the same tumor.

Methods: Overall, 90 cases comprising 47 lung, 11 breast, 9 colon, 6 stomach, and 7 pancreatic carcinomas and 10 other tumors were included in the study. Of these, 43 specimens were obtained by resection, 40 by tru-cut biopsy, and 7 by endoscopic biopsy. Sections prepared from formalin-fixed paraffin-embedded blocks were evaluated immunohistochemically with SP142 and SP263 clones.

Results: In this study, we observed positive staining in 48.8% (n=44) and negative staining in 51.2% (n=46) among all cancers with SP263 clone, and positive staining in 33.3% (n=30) and negative staining in 66.7% with SP142 clone as well. This study also showed that compared to SP263, SP142 clone stained tumor cells less in lung, colon, stomach, pancreatic, and other carcinomas.

Conclusion: In this study, we found different staining percentages for SP263 and SP142 in the same tumor. Pathologists conducting immunohistochemical studies for PD-L1 should indicate the staining percentages of tumors and the antibody clone they used in the reports. Meanwhile, oncologists should keep in mind which clone was stained, and that selecting SP142 is less positive to correct patients who can receive appropriate immunotherapy.

Keywords: Cancer, immunohistochemistry, staining, stomach
PD-L1 expression on tumor cells has been investigated for melanoma and in tumor cells of breast, lung, colorectal, and pancreatic cancers. Due its potential for toxic effects and higher costs of using PD-1/PDL1 inhibitors, the patient selection has become an important issue. Several studies have shown that there is a significant relationship between the level of PD-L1 expression detected in the immunohistochemical analysis in formalin-fixed paraffin-embedded tissue samples and the response rates for immunotherapy and results. The detection of PD-L1 release in tumor cells and immune cells by the immunohistochemical method has been used as a biomarker and indicator of immunotherapy use and efficacy. There are many subclones for PD-L1 antibody (22C3, SP263, 28-8, SP142, E1L3N). Antibodies specific to each drug validated immunohistochemical platforms, and different positivity percentages were determined for each antibody. However, this leads to inconsistency in the evaluations of pathologists, difficulties in practical results, and higher costs for pathology departments.

The aim of this study was to compare two different PD-L1 antibody clones in terms of expression by using validated immunohistochemical platforms among paraffin-embedded tissue samples of a heterogeneous group with breast, colon, stomach, and pancreatic carcinomas.

Methods

Materials
Paraffin-embedded tumor specimens of 90 cases were collected by an experienced pathologist, and serial slides were prepared to cover the entire surface. Overall, 47 of the cases were lung cancer, 11 breast cancer, 9 colon cancer, 6 stomach cancer, 7 pancreatic carcinoma, and the rest 10 various tumors. Of these specimens 43 were obtained by resection, 40 by thick needle biopsy, and 7 by endoscopic biopsy.

Immunohistochemical Examination and Evaluation
We obtained serial slides of 4-µm thickness covering the entire surface from paraffin-embedded tumor blocks fixed with formaldehyde. Both placenta and tonsil tissue were used for each slide as positive control tissue. Two different antibody clones against PD-L1, SP142 (Ventana Medical Systems) and SP263 (Ventana Medical Systems) were studied on an automatized Ventana staining platform with the Ventana Benchmark Ultra OptiView Universal DAB kit. Immunohistochemical staining was performed with Benchmark GX immunoaumtate (Ventana Medical Systems Inc, Tucson, USA), OptiView DAB IHC Detection Kit and OptiView Amplification Kit (Ventana Medical Systems Inc, Tucson, USA). All stages were completed in accordance with standard and validated immunohistochemical protocols.

All hematoxylin-eosin stained prepares and immunohistochemically stained prepares were evaluated for PD-L1 expression by a trained and experienced pathologist. Partial or complete membranous staining of tumor cells was considered positive staining. The percentage of stained tumor cells was defined. Immune cells and necrotic areas were not evaluated.

Statistical Analysis
The statistical analyses with chi-square test, Friedman's test, and McNemar's test were carried out using SPSS software.

Results
Overall, 90 tumor cases were included in this study. Of these, 47 were lung, 11 were breast, 9 were colon, 6 were stomach, and 7 were pancreatic carcinoma and the rest 10 were various tumors. The median age was 61 (28–81) years.

| Table 1. Descriptive statistics for demographic and clinicopathological features of the cases (n=90) |
|---|---|---|
| **Factors** | **n** | **%** |
| Age (years) | | 61 (28–81) |
| ≤60 | 43 | 47.8 |
| >60 | 47 | 52.2 |
| Sex | | |
| Male | 57 | 63.3 |
| Female | 33 | 36.7 |
| Tumor site | | |
| Lung | 47 | 52.2 |
| Breast | 11 | 12.2 |
| Colon | 9 | 10.0 |
| Stomach | 6 | 6.7 |
| Pancreas | 7 | 7.8 |
| Other | 10 | 11.1 |
| Histological tumor type | | |
| Adenocarcinoma | 52 | 57.8 |
| Squamous cell carcinoma | 17 | 18.9 |
| Invasive ductal carcinoma | 11 | 12.2 |
| Other | 10 | 11.1 |
| Biopsy type | | |
| Tru-cut | 40 | 44.4 |
| Resection | 43 | 47.8 |
| Other (excisional, endoscopy) | 7 | 7.8 |
| SP-142 | | |
| Positive | 30 | 33.3 |
| Negative | 60 | 66.7 |
| SP-263 | | |
| Positive | 44 | 48.9 |
| Negative | 46 | 51.1 |
features of the cases. Staining in ≥1% of tumor cells were considered PD-L1 positive. Positive staining was observed in 48.8% (n=44) and negative staining in 51.2% (n=46) of all cases with the SP263 clone. With the SP142 clone, positive staining was observed in 33.3% (n=30) and negative staining in 66.7%. In 14 of the cases that stained positive with SP263, negative staining with SP142 was observed. Table 2 and Table 3 present the positive and negative staining ratios of the two clones (SP263 and SP142). Differentiation was observed between the two clones in terms of immunohistochemical staining percentage and staining intensity in sections belonging to the same tumor. Although SP142 showed less intense and lighter staining on tumor cells, SP263 showed more intense and darker staining on tumor cells (Figs. 1, 2). There was a heterogeneous staining pattern in tumor cells for both clones (Fig. 3).

**Discussion**

The evaluation of PD-L1 expression on tumors as immunohistochemical analysis has recently been used frequently for anti-PD1 and anti-PD-L1 treatment options. In this study, we investigated immunohistochemical staining of PD-L1 expression in two different clones (SP142 and SP263) for 90 tumor cases. In the literature, there has been a large body of research evaluating PD-L1 expression with various clones on different immunohistochemical platforms.[8, 9] In the Blueprint 1 study conducted by Hirsch et al.,[7] four different immunohistochemical tests (22C3, 28–8, SP142, SP263) were evaluated in paraffin blocks consisting mostly resection material diagnosed with non–small-cell lung carcinoma. In the Blueprint 2 project, five different immunohistochemical tests were evaluated on 81 lung carcinoma specimens from various histological and biopsy sample types.[10] Moreover, in the German harmonization study, 15

<table>
<thead>
<tr>
<th>Test value</th>
<th>SP-142 n (%)</th>
<th>SP-263 n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>30 (33.3)</td>
<td>44 (48.9)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Negative</td>
<td>60 (66.7)</td>
<td>46 (51.1)</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05; McNemar’s test.

<table>
<thead>
<tr>
<th>Test</th>
<th>Percentage of positivity (%)</th>
<th>Mean and standard deviation</th>
<th>X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP-142</td>
<td>35.83±32.42</td>
<td>41.000</td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SP-263</td>
<td>46.20±37.19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05; X²=Friedman’s test.

**Figure 1.** (a) PD-L1 SP142 immunohistochemical staining in tru-cut biopsy. (b) PD-L1 SP263 immunohistochemical staining in tru-cut biopsy.

**Figure 2.** (a) PD-L1 SP142 immunohistochemical staining in resection specimen. (b) PD-L1 SP263 immunohistochemical staining in resection specimen.
l lung carcinoma resection specimens were studied on PD-L1 expression with again in 28–8 and 22C3 clones in tumor cells and 28–8, 22C3, SP263 clones with SP142 clone were found to be less than 28–8, 22C3, with SP263 clone. They detected greater expression than SP142 clones. Similar results for SP263, 22C3, and 28-8 were presented by Ratcliffe MJ et al. in a study conducted with 493 lung carcinoma cases. Rimm DL et al., also studied 28–8, 22C3, SP142, and E1L3N clones in 90 lung carcinoma cases. They found lower PD-L1 levels with SP142 compared to other clones in tumor and immune cells. Similar results were obtained in another study. In the study conducted by Parra ER et al., in 259 lung cancer cases, using microarray method with different clones (E1L3N, E1J2J, SP142, 28-8, SP263, 5H1), the positively stained case was mostly SP263 and then SP142, E1J2J, respectively. 28–8, 22C3, E1L3N were detected and found to express SP142 more than other clones, unlike the literature. This can be explained by the fact that the tissues were studied in a small area using the microarray method. In this study, we found different staining percentages for SP263 and SP142 in the same tumor. Positive staining was detected in 48% of all carcinomas with SP263 and in 33% of all carcinomas with PS142 when the whole tumor area on the preparates was evaluated. As a histological subtype, both clones had a higher positivity ratio in squamous cell carcinoma compared to adenocarcinomas (Tables 4, 5).

Numerous studies have focused on lung cancers. Despite the small sample size, epithelial cancers in various organs were also included in our study. All epithelial tumors, including those of colon, stomach, and pancreas, showed lower staining level with SP142, whereas breast carcinomas showed higher positivity for SP142 in both clones. However, there were only 11 cases of breast tumor included in this study; therefore, studies with larger number of series are needed.

As a result, in accordance with the literature, this study shows that SP142 has less positivity (≥1%) on lung cancers. To our knowledge, there is less positivity with SP142

### Table 4. Findings for SP-142 by the demographic and clinicopathological features of the cases

<table>
<thead>
<tr>
<th>Factors</th>
<th>Positive</th>
<th>Negative</th>
<th>X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤60</td>
<td>15 34.9</td>
<td>28 65.1</td>
<td>0.089</td>
<td>0.765</td>
</tr>
<tr>
<td>&gt;60</td>
<td>15 31.9</td>
<td>32 68.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21 36.8</td>
<td>36 63.2</td>
<td>0.861</td>
<td>0.353</td>
</tr>
<tr>
<td>Female</td>
<td>9 27.3</td>
<td>24 72.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>21 44.7</td>
<td>26 55.3</td>
<td>11.503</td>
<td>0.042*</td>
</tr>
<tr>
<td>Breast</td>
<td>2 18.2</td>
<td>9 81.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>0 0.0</td>
<td>9 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>1 16.7</td>
<td>5 83.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>1 14.3</td>
<td>6 85.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 50.0</td>
<td>5 50.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histological tumor type</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>15 28.8</td>
<td>37 71.2</td>
<td>4.749</td>
<td>0.191</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>9 52.9</td>
<td>8 47.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>2 18.2</td>
<td>9 81.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>5 50.0</td>
<td>5 50.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05; X²=Chi-square test.

### Table 5. Findings for SP263 by the demographic and clinicopathological features of the cases

<table>
<thead>
<tr>
<th>Factors</th>
<th>Positive</th>
<th>Negative</th>
<th>X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤60</td>
<td>21 48.8</td>
<td>22 51.2</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>&gt;60</td>
<td>23 48.9</td>
<td>24 51.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>33 57.9</td>
<td>24 42.1</td>
<td>5.046</td>
<td>0.025*</td>
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<tr>
<td>Female</td>
<td>11 33.3</td>
<td>22 66.7</td>
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<tr>
<td>Tumor site</td>
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</tr>
<tr>
<td>Lung</td>
<td>30 63.8</td>
<td>17 36.2</td>
<td>13.600</td>
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<tr>
<td>Breast</td>
<td>2 18.2</td>
<td>9 81.8</td>
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<tr>
<td>Colon</td>
<td>1 11.1</td>
<td>8 88.9</td>
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<td>Stomach</td>
<td>3 50.0</td>
<td>3 50.0</td>
<td></td>
<td></td>
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<tr>
<td>Pancreas</td>
<td>3 42.9</td>
<td>4 57.1</td>
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<tr>
<td>Other</td>
<td>5 50.0</td>
<td>5 50.0</td>
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<tr>
<td>Histological tumor type</td>
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<tr>
<td>Adenocarcinoma</td>
<td>27 51.9</td>
<td>25 48.1</td>
<td>6.361</td>
<td>0.095</td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>11 64.7</td>
<td>6 35.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>2 18.2</td>
<td>9 81.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 40.0</td>
<td>6 60.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05; X²=Chi-square test

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**Figure 3.** Heterogeneous staining.
on colon, stomach, and pancreatic cancers, whereas breast carcinomas are stained equally in both clones. In our research, the positivity level of PD-L1 was accepted at ≥1% for all other tumors except lung cancers as indicated in the lung cancer guidelines. Pathologists conducting immunohistochemical studies for PD-L1 should specify the staining percentages of tumors and the antibody clone they used in the reports. Meanwhile, oncologists should remember which clone was stained and SP142 is less positive.

Disclosures

Ethics Committee Approval: The ethics committee of Acibadem University provided the ethics committee approval for this study (19/12/2019-2019/20).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

References


